

AMENDED SET OF CLAIMS

1. (Previously Presented) A method for purifying a calcium ion-binding protein from a sample containing said protein using a cation exchange carrier, wherein said method comprises

contacting the sample with the cation exchange carrier in the presence of calcium ions to let the protein be adsorbed to the exchange carrier; and

eluting the adsorbed calcium ion-binding protein from the exchange carrier by

(a) decreasing or removing the concentration of said calcium ions

(b) adding counter ions other than said calcium ions or

(c) both (a) and (b).

2. (Original) The method of claim 1 wherein the adsorption step is performed in the presence of 5 to 100 mM calcium ions.

3. (Original) The method of claim 2 wherein the adsorption step is performed in the presence of 10 to 30 mM calcium ions.

4. (Original) The method of any one of claims 1 to 3 wherein the adsorption step is performed at a flow rate of 1 to 150 cm/h.

5. (Original) The method of claim 4 wherein the adsorption step is performed at a flow rate of 15 to 100 cm/h.

6. (Original) The method of claim 4 wherein the adsorption step is performed at a flow rate of 50 to 80 cm/h.

7. (Previously Presented) The method of claim 1 wherein the elution step is performed by decreasing the concentration of calcium ions to less than 5 mM.

8. (Original) The method of claim 1 wherein the elution step is performed by adding 1 to 500 mM of counter ions other than calcium ions.

9. (Original) The method of claim 1 wherein the elution step is performed by adding 50 to 500 mM of counter ions other than calcium ions.

10. (Original) The method of claim 1 wherein the elution step is performed by adding 50 to 300 mM of counter ions other than calcium ions.

11. (Original) The method of claim 1, 8, 9 or 10 wherein said counter ions are selected from the group consisting of Na^+ , Li^+ and K^+ .

12. (Previously Presented) The method of claim 1, 7, 8, 9 or 10 wherein the elution step is performed at a flow rate of 1 to 150 cm/h.

13. (Original) The method of claim 12 wherein the elution step is performed at a flow rate of 30 to 100 cm/h.

14. (Original) The method of claim 12 wherein the elution step is performed at a flow rate of 30 to 80 cm/h.

15. (Original) The method of claim 1 wherein the cation exchange carrier is selected from the group consisting of SP-Sepharose, CM-Sepharose, CM-cellulose, SE-cellulose, S-Spherodex and SP-Spherosil.

16. (Previously Presented) The method of claim 1 wherein the calcium ion-binding protein is selected from the group consisting of Annexins I, II, III, IV, V, VI and VII.

17. (Previously Presented) The method of claim 1 wherein the sample contains a calcium ion-binding protein prepared by genetic recombination.

18. (Previously Presented) The method of claim 1 wherein the adsorption and elution steps are performed at pH 5 to 10.

19. (Original) The method of claim 18 wherein the adsorption and elution steps are performed at pH 8 to 9.5.

20. (Original) The method of claim 19 wherein the adsorption and elution steps are performed at pH 9.

21. (Previously Presented) The method of claim 1 wherein
the adsorption step is performed in the presence of 10 to 30 mM calcium ions at pH 8 to 9.5 at a flow rate of 15 to 100 cm/h;
the elution step is performed at a flow rate of 30 to 80 cm/h by decreasing the concentration of calcium ions to less than 5 mM or by adding 50 to 300 mM counter ions selected from the group consisting of Na⁺, Li⁺ and K⁺;
the cation exchange carrier is SP-Sepharose;
the calcium ion-binding protein is Annexin V;
the sample contains Annexin V prepared by genetic recombination; and
protease is removed from the sample.

22. (Previously Presented) The method of claim 1 wherein
the adsorption step is performed in the presence of 10 to 30 mM calcium ions at pH 8 to 9.5 at a flow rate of 15 to 100 cm/h;

the elution step is performed at a flow rate of 30 to 80 cm/h by decreasing the concentration of calcium ions to less than 5 mM or by adding 500 mM counter ions selected from the group consisting of Na^+ , Li^+ and K^+ ;

the cation exchange carrier is SP-Sepharose;

the calcium ion-binding protein is Annexin VI;

the sample contains naturally occurring Annexin VI; and

protease is removed from the sample.

23. (Original) A method for purifying a calcium ion-binding protein from a sample containing said protein using a cation exchange carrier, wherein said method comprises contacting the sample with the cation exchange carrier in the presence of calcium ions to let the protein be adsorbed to the carrier.

24. (Original) The method of claim 23 wherein said method is performed in the presence of 5 to 100 mM calcium ions.

25. (Original) The method of claim 24 wherein said method is performed in the presence of 10 to 30 mM calcium ions.

26. (Original) The method of any one of claims 23 to 25 wherein said method is performed at a flow rate of 1 to 150 cm/h.

27. (Original) The method of claim 26 wherein said method is performed at a flow rate of 15 to 100 cm/h.

28. (Original) The method of claim 26 wherein said method is performed at a flow rate of 50 to 80 cm/h.

29. (Original) The method of claim 23 wherein the cation exchange carrier is selected from the group consisting of SP-Sepharose, CM-Sepharose, CM-cellulose, SE-cellulose, S-Spherodex and SP-Spherosil.

30. (Previously Presented) The method of claim 23 wherein the calcium ion-binding protein is selected from the group consisting of Annexins I, II, III, IV, V, VI and VII.

31. (Previously Presented) The method of claim 23 wherein the sample contains a calcium ion-binding protein prepared by genetic recombination.

32. (Previously Presented) The method of claim 23, wherein the method is performed at pH 5 to 10.

33. (Original) The method of claim 32 wherein the method is performed at pH 8 to 9.5.

34. (Original) The method of claim 33 wherein the method is performed at pH 9.

35. (Previously Presented) A calcium ion-binding protein selected from the group consisting of Annexins I, II, III, IV, V, VI and VII having a purity of 80 to 100% as determined by gel filtration chromatographic analysis, obtained by the method of claim 1.

36. (New) A calcium ion-binding protein selected from the group consisting of Annexins I, II, III, IV, V, VI and VII having a purity of 80 to 100% as determined by gel filtration chromatographic analysis or SDS-PAGE analysis, obtained by the method of claim 1.

37. (New) A calcium ion-binding protein selected from the group consisting of Annexins I, II, III, IV, V, VI and VII having a purity of 80 to 100% as determined on a molecular size basis, obtained by the method of claim 1.